Elytra, *Tokyo*, New Series, **9** (2): 279–283
December 25, 2019

Ecological Notes on *Laccophilus nakajimai* Kamite, Hikida et Satô, 2005 (Coleoptera, Dytiscidae)

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Abstract *Laccophilus nakajimai* Kamite, Hikida et Satô, 2005 collected from Yonaguni-jima Island were reared in the laboratory. Eggs could not be confirmed in this laboratory rearing experiment, but it is likely that *L. nakajimai* laid eggs into plant, *Blyxa japonica* (Hydrocharitaceae). Ten larvae were hatched. All larval stages of *L. nakajimai* are illustrated for the first time. The developmental period for each stage was as follows: 1st instar larva, two days; 2nd instar larva, three to seven days; 3rd instar larva, six to 14 days; period from landing to escaping from pupal chamber, which includes pupation and emergence, eight to twelve days. It is likely that the reproductive season of *L. nakajimai* is at least from May to June because a 3rd instar larva was collected on June 16 in the natural environment and the total period of the immature stage was 23 to 26 days excluding egg stage. The 3rd instar larvae made pupal chambers at the highest place on the soil in the pupation cups. It is possible that selecting a relatively high position for the pupal chamber may help to avoid flooding owing to rising water levels because *L. nakajimai* inhabits small streams and the rainy season overlaps with their reproductive season.

Introduction

Laccophilus nakajimai Kamite, Hikida et Satô, 2005 (Fig. 1A) is a small dytiscid species belonging to the *Laccophilus kobensis* species group and inhabits small streams (Kamite *et al*., 2005). This species has only been recorded from Yonaguni-jima Island in the Ryukyus, Japan and is listed as "vulnerable" in the current national Red List of Japan (Ministry of the Environment of Japan, 2018). However, little is known about its life history (Ministry of the Environment of Japan, 2015), particularly during immature stages. Discovering larvae will contribute to elucidating its seasonal prevalence and life history characteristics in a natural environment and conservation.

I attempted to rear *L. nakajimai* in the laboratory to reveal the ecology of its immature stages. In the present paper, all larval stages of *L. nakajimai* have been illustrated for the first time. Additionally, I reported the larval record of *L. nakajimai* in the natural environment.

Materials and Methods

More than ten individuals of *Laccophilus nakajimai* adults were collected at Maganda, Yonaguni-chô, Yonaguni-jima Island, Okinawa Prefecture, Japan on June 17, 2018. Ten of them were brought to the rearing room at the Ishikawa Insect Museum, Hakusan, Ishikawa Prefecture, Japan on June 20, 2018. The rearing room was maintained at 26 ℃ with nine hours of light (from 8:15 AM to 5:15 PM) and 15 hours of darkness (9L : 15D). I placed Java moss (Hypnaceae) and *Blyxa japonica* (Hydrocharitaceae) in a plastic rearing cup for adults (13 cm in diameter, 6 cm in height) to provide sites for hiding. Adequate quantities of frozen chironomid larvae were provided to adults as prey every two to three days. The rearing experiment was terminated on January 11, 2019.

The rearing container was checked every one to three days. When eggs or larvae were found, I

recorded the date of confirmation and developmental stage. The larvae were transferred into plastic cups (called larval cups, 8 cm in diameter, 4 cm in height, ca. 5 mm in water depth) containing Java moss to rear individually. The all larvae were fed living chironomid larvae collected from the pond at the Ishikawa Insect Museum. I prepared another plastic cups (called pupation cups, the same size as larval cups) with crushed and moistened peat moss (1 cm in depth) for the landing soil. I carefully placed 3rd instar larvae on the peat moss in the pupation cups when they stopped eating prey items and walked without stopping. The insects were reared below 26 ℃. The dates of molting, transition to the soil, and escapement of newly emerged adults to the soil surface and the conditions of individuals were recorded in detail. Several individuals were placed in ethanol to retain as specimens, and one pupal chamber was dissected for observation.

Field survey was conducted to collect the larvae in a small stream in Urabu, Yonaguni-chô, Yonaguni-jima Island on June 16, 2018. Identification was made by comparison with larvae obtained by the rearing experiments.

Photographs were taken using a Nikon D500 digital camera equipped with Nikon AF-S VR MICRO-NIKKOR 105 mm f/2.8G ED and Raynox DCR-250 or Kenko EXTENSION TUBE (36, 20, and 12 mm). The images were stacked with digital image processing software Zerene Stacker version 1.04 (Zerene Systems LLC, USA) for Fig. 1G.

Results

Two 2nd instar larvae were obtained on November 5, 2018, 138 days after the start of adult rearing. Eggs were not be found. In total, ten larvae (five 1st instar and five 2nd instar larvae) were obtained until December 16, 2018. The period of each developmental stage was as follows: total larval period, 13 to 17 days (mean ± SD = 14.7 ± 1.7 days, n = 3); 1st instar (Fig. 1B), two days (2.0 ± 0.0 days, n = 4); 2nd instar (Fig. 1C–D), three to seven days (4.6 ± 1.3 days, n = 7); 3rd instar (Fig. 1E–F), six to 14 days (9.8 ± 2.9 days, n = 6) (Table 1). The larvae ate living chironomid larvae as prey (Fig. 1C). The 3rd instar larval body became green before landing (Fig. 1F). After transferring to land, the larvae walked on the soil surface. The larvae chose the highest place on the soil and made pupal chambers on the soil surface. Pupation took place in pupal chambers. I observed a pupa by dissecting a pupal chamber three days after it was made (Sample No. 5, Table 1, Fig. 1G), because the inside of the pupal chamber could not be seen in all pupation cups. The size of the pupal chamber of Sample No. 5 was 4.63 mm long and 4.38 mm wide. Emergence was observed seven days (n = 1) after the pupal chamber was made (Fig. 1H). The period from landing to escaping was eight to twelve days (9.8 ± 1.3 days, n = 5). All newly emerged adults escaped from the pupal chambers to the soil surface. These adults began to feed on frozen chironomid larvae from the day they escaped from the pupal chambers. The total period of the immature stages excluding egg stage was 23 to 26 days (25.0 ± 1.4 days, n = 3).

I collected a similar larva in the natural environment. It was identified as a 3rd instar of the same species by morphological comparison with the reared larvae.

Discussion

Laccophilus nakajimai eggs could not be found in this laboratory rearing experiment. The egg-laying behaviors of dytiscid beetles are diverse: gluing eggs to surfaces of aquatic plants or other objects, dropping randomly or placing eggs on oviposition substrate, and inserting eggs into plants (e.g., ICHIKAWA, 2002; INODA, 2011; MILLER & BERGSTEN, 2016; WATANABE *et al*., 2017). The author

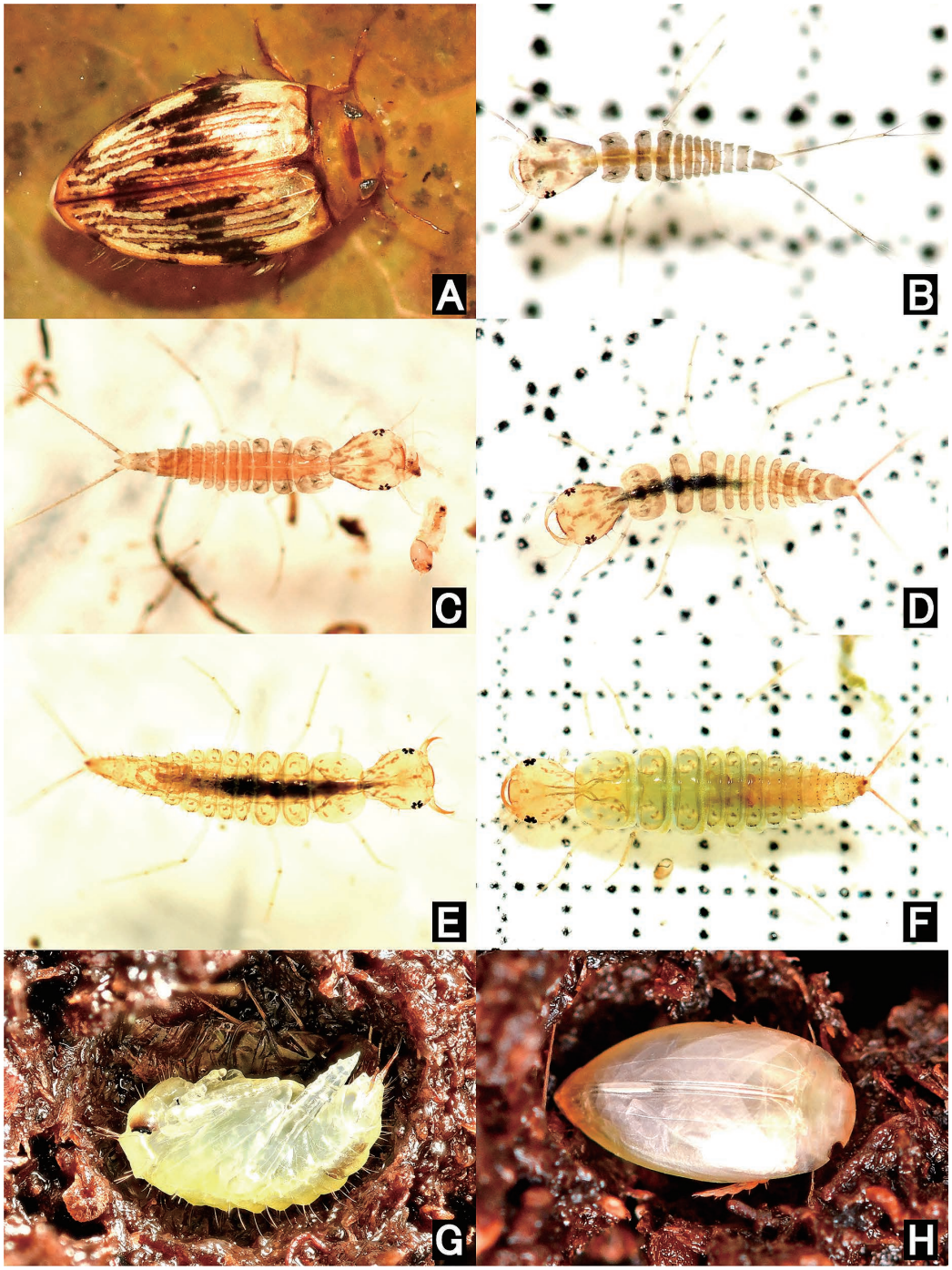

Fig. 1. *Laccophilus nakajimai*. —— A, Adult obtained from Yonaguni-jima Island; B, 1st instar larva; C, 2nd instar larva preying on a living chironomid larva; D, 2nd instar larva; E, 3rd instar larva; F, 3rd instar larva immediately before landing; G, pupa in a pupal chamber; H, new adult immediately after emergence. Edge length of a grid mesh in B, D & F: 1.0 mm.

Table 1. The period for each developmental stage of *Laccophilus nakajimai*.

Sample No.	Period (days) of each larval stage and that from landing to escaping				Note
	1st instar	2nd instar	3rd instar	Landing to escaping	
1	●	–	–	–	Artificially killed
2	–	●	–	–	Artificially killed
3	–	3	●	–	Artificially killed
4	–	3	14	10	
5	–	7	13	8	⁎
6	2	●	–	–	Naturally died
7	–	4	9	●	Naturally died
8	2	5	6	10	
9	2	5	10	9	
10	2	5	7	12	
Mean ± SD	2.0 ± 0.0 (n = 4)	4.6 ± 1.3 (n = 7)	9.8 ± 2.9 (n = 6)	9.8 ± 1.3 (n = 5)	

⁎: The pupal chamber was destroyed three days after the formation.
●: Black circle represents that an individual died at the corresponding developmental stage.

observed that eggs of *Laccophilus difficilis* SHARP, 1873 were laid into *Blyxa japonica* (WATANABE, unpublished data). It is likely that *L. nakajimai* also laid eggs into *B. japonica* in this experiment.

In the natural environment, a 3rd instar larva was found on June 16, 2018. As the total larval period was 13 to 17 days in this study, it is possible that the reproductive season of *L. nakajimai* is at least from May to June.

When 3rd instar larvae made pupal chambers, they chose the highest place on the soil in the pupation cup. *Laccophilus nakajimai* inhabits small streams in Yonaguni-jima Island (KAMITE *et al*., 2005; Ministry of the Environment of Japan, 2015). Water levels of rivers and streams can easily increase with rainfall. The rainy season called "Tsuyu" begins from about May to June in Yonaguni-jima Island (Okinawa Regional Headquarters, 2018), which overlaps with the reproductive season of *L. nakajimai*. Therefore, it is possible that making the pupal chambers in high positions may avoid flooding owing to rising water levels.

The larval stages of *Laccophilus obliquatus* and *L. paraguensis* can be distinguished by the color pattern of the head (MICHAT, 2008). It is considered that the larval stages of *L. nakajimai* can be identified by the following color pattern of dorsal aspect of the head: 1st instar larvae have a brown Y-shaped vitta at the center and a single spot on each side (Fig. 1B), 2nd instar larvae have two light brown posterior spots on each ocular region (Fig. 1C–D), and 3rd instar larvae have some light brown vittae from the basal to posterior area on each ocular region (Fig. 1E–F). However, in the present study, sufficient samples for adequate descriptions were not obtained because the priority was given to the elucidation of life history characteristics. To determine useful morphological characteristics for distinguishing among larval stages and species, further researches including morphological descriptions are required.

Acknowledgements

I express my sincere gratitude to Dr. Yûsuke N. MINOSHIMA (Kitakyushu Museum of Natural History and Human History) for critically reading the manuscript; Mr. Masataka SUDA and Mr. Wataru YOSHIDA at the Ishikawa Insect Museum for their cooperation with rearing experiments and con-

firming larval stages; and the entire staff of the Ishikawa Insect Museum for great cooperation during this study. Finally, I would like to thank Editage (www.editage.jp) for English language editing.

要　　約

渡部晃平：ナカジマツブゲンゴロウ（鞘翅目ゲンゴロウ科）の生態的知見．――――本研究では，ナカジマツブゲンゴロウ *Laccophilus nakajimai* Kamite, Hikida et Satô, 2005 の繁殖生態を解明することを目的として飼育実験を行った．飼育下において 10 頭の幼虫が得られた．各発育段階における成育期間は 1 齢幼虫（2 日），2 齢幼虫（3~7 日），3 齢幼虫（6~14 日），上陸~羽化脱出（8~12 日）であった．卵の確認には至らなかったが，ヤナギスブタの茎の中に産卵したものと推測された．自然環境下において 3 齢幼虫が 6 月 16 日に採集されたこと，実験より得られた幼虫および上陸後の累計成育日数（23~26 日）から，少なくとも 5~6 月が本種の繁殖期に含まれると考えられた．上陸が近づいた 3 齢幼虫は土の最も高いところで土繭（蛹室）を作った．細流に生息する本種の繁殖期が梅雨と重なることで，蛹室が増水にさらされる頻度が高まる可能性があることから，高い位置に蛹室を作ることは浸水を避けるために役立つものと思われる．

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Manuscript received 4 August 2019;
revised and accepted 15 November 2019.